USE OF DEPILATORY AGENT IN PREPARATION OF MOUSE FOOT PAD CELL CULTURE *

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ABSTRAK

Telah dijelaskan secara rinci bagaimana cara pembuatan biakan sel telapak kaki (Mouse Foot Pad Cell Culture) mencit C 3 H. Kunci keberhasilan pembuatan ini terletak pada penggunaan bahan penghilang rambut (depilatory agent) sebagai pembersih bulu telapak kaki, sehingga tidak terjadi kontaminasi kuman dan menggunakan sel medium campuran 109–199.

Biakan sel ini telah berumur lebih dari 20 tahun dan masih dipergunakan di Bagian Bakteriologi, Fakultas Kedokteran, Universitas Hiroshima, Japan oleh Prof. Y. Matsuo sebagai substrat dalam penelitian *Mycobacterium lepraemurim*. Menurut Prof. Matsuo jenis biakan sel ini merupakan satu-satunya yang ada di dunia dan merupakan salah satu substrat yang terbaik untuk penelitian penyakit Hansen.

INTRODUCTION

Between 1950 and 1960, there were several reports in which multiplication of *Mycobacterium lepraemurium* had been observed in cultures of mouse and rat spleen explants and of peritoneal macrophages of mouse and rat as well as in established cell line cultures[1–6]. *M. lepraemurium* and *M. leprae* have been successfully cultured in vivo in mouse foot pads, however, no published report had yet been known on using mouse foot pad (MFP) cell culture for growing the same organism in establishing the MFP line. According to DR. Matsuo, Department of Bacteriology, School of Medicine, Hiroshima University, Japan and DR. Kirchheimer, U.S. Public Health Service Hospital, Carville Louisiana, USA., (personal communications), they could not grow this cell line because of the consistent contamination with bacteria within 2 week incubation period. They could not completely sterilize the contaminated foot after shaving and washing with pHisohex, iodine tincture, 70 % alcohol and dipping the amputated foot in antibiotics solution for disinfection.

The present paper describes the simple technique of using depilatory agent in preparation of MFP cell culture and discuss the success of establishing this cell line.

MATERIALS AND METHODS

I. Source of Tissue: Fifteen adult C 3H mice of both sexes were used for the preparation of foot pad cell culture.

II. Composition of Medium: The medium used in this tissue culture is the 109–199 medium and its composition is as follows :

1. Medium 109 . . . . . .200.00 ml
2. Medium 199 . . . . . .20.00 ml
3. Fetal calf serum. . . . . .40.00 ml

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* This study was undertaken between 1966–67 when the author was taking his Master Degree of Science at the Department of Pathology, Purdue University, West Lafayette, Indiana, U.S.A.

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4. L—glutamine.....2.00 ml
5. Fungizone.....0.40 ml
6. Penicillin + streptomycin. .1.00 ml
7. Triple distilled water ...180.00 ml

III. Procedure: The preparing of the foot pad cells is as follows:

1. Remove the hair around the hind legs and the foot pads with depilatory agent (Blue Magic shaving Powder, Carson Chemical Co., Savannah, GA. U.S.A.). Prepare the powder by mixing with tap water until it becomes a thick solution and smear the hind legs thoroughly. Then wait for about 3 minutes and remove the hair completely by wasing with tap water.
2. Scrub the hairless legs and the foot pads (FP) with pHisohex and then wash with tap water thoroughly.
3. Swab the hind legs and FP with iodine tincture 1%.
4. Wash and clean the iodinated legs with 70% alcohol so that all the iodine is completely removed.
5. Kill the animal by cervical luxation, and then cut the limbs and dip them into tissue culture medium containing 5 x strength penicillin and streptomycin for 15 menutes.
6. Remove the foot pad from the limb and mince it with scissors into very small pieces.
7. Put the minced tissue into a fluted flask with magnetic bar and add Hank's BSS solution.
8. Stir it in the incubator for 10 minutes and repeat twice, so that no more red blood cells left in the tissue. Discard the supernatant.
9. Add trypsin 0,2% in proportion 1 : 10, and stir it in the incubator (35C) for 15 minutes, and harvest the cells by passing the supernatant through a sterile gauze. Repeated 3—4 times.
10. Centrifuge the supernatant in cold (referigerated centrifuge) at 2.000 rpm for 5 minutes.
11. Discard the supernatant.
12. Disperse the residue in the tissue culture medium.
13. Distribute in tissue culture flask and incubated at 37° C.
14. Change the medium after 48 hours, and then change the medium at weekly intervals.

The use of the simple technique as described above by using depilatory agent as the "Shaving" method is the most effective and effecient one, since it not only removes the hair shafts (like the razor blade does) but at the same time it denudes completely the entire layer of stratum corneum and hair follicles. These two areas seemed to be the notorious hiding places of the microorganisms and have shown to be the sources of consistent contamination experienced by Prof. Matsuo and DR. Kirchhiemer.

The author also experienced several failures. It was not because of contamination, but rather due to unsuitable media. For instance, medium which contained MEM, 10% fetal calf serum, and yeastolate did not support the cell growth beyond 2 weeks observation. Failure also experienced if the medium contained the following ingridients:

1. Hank solution (10x) ..... .10.00 ml
2. Phenol Red 1% .....0.10 ml
3. MEM amino acid (50x) .....2.00 ml
4. Vitamins (100x) ....1.00 ml
5. L—glutamine..... ..... 1.00 ml
6. Fungozone ....0.10 ml
7. Penicillin and streptomycin. . .1.00 ml

8. Na H CO_3 7.5% .....1.50 ml
9. Non essential amino acids ....1.00 ml
10. Aqua tridestilated ...ad . .100.00 ml
11. Fetal calf serum.....10.00 ml

The medium 109—199 has shown to be most suitable for growing rodent tisssues according to DR. D. Byington. This MFP cell culture has been maintained since 1967. This cell is still alive and is kept at the Department of Bacteriology, School of Medicine, Hiroshima University, Hiroshima, Japan. DR. Matsuo has success fully used this cell line for the last 20 years for his research in Hansen disease[7-11].

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REFERENCES

1. Rees. R.J.W. and Wong, P.C. (1959) : Limited multiplication of *Mycobacterium lepraemurium* in tissue culture. *Nature*. 181 : 359—360.
2. Wallace, J.H., Elck, S.K. and Hanks, J.H. (1958) : Limited multiplication of *Mycobacterium lepraemurium* in cell cultures. *Proc. Soc. Exp. Biol. Med.* 97 : 101—104.
3. Rees, R.J.W.and Garbutt, E.W. (1962): Studies on *Mycobacterium lepraemurium* in tissue culture. I. Multiplication and growth characteristics in cultures of rat fibroblasts. *Brit. J. Exp Path* 43 : 221—235.
4. Garbutt, E.W., Rees, R.J. W. and Barr, Y.M. (1962): Growth of *Mycobacterium lepraemurium* maintained in cultures of rat fibroblasts. *J. Gen. Microbiol.* 27 : 259—268.
5. Chang, Y.T. and Neikirk, R.L. (1965): *Mycobacterium lepraemurium* and *Mycobacterium leprae* in cultures of mouse peritoneal macrophages. Internat. *J. Leprosy* 33: 586—598.
6. Chang Y.T., Anderson, R.N. and Vaituzis, Z. (1967) : Growth of *Mycobacterium lepreamurium* in cultures of mouse peritoneal macrophages. *J. Bacteriol*.93 : 1119—1131.
7. Matsuo, Y. (1970) : Multiplication of *Mycobacterium lepraemurium* in mouse foot pad cell culture. *Japan J. Microbiol.* 14 : 233—240.
8. B. Matsuo, Y. (1974) : Studies of *Mycobacterium lepraemurium* in cell culture. I. Continuous multiplication in cultures of mouse foot pad cells. *Japan. J. Microbiol.* 18 : 307—312.
9. Matsuo, Y. (1975) : Studies of *Mycobacterium lepraemurium* in cell culture. II. Pathogenicity of *Mycobacterium lepraemurium* maintained in mouse foot pad cell culture and in teraction of the bacilli with the infected cells. *Japan J. Microbiol.* 19 : 319 — 325.
10. Matsuo, Y. and Utsunomiya, S. (1976) : Attempts at cultivation

of *Mycobacterium leprae* in cell cultures. *Japan J. Microbiol.* 20 : 471 – 473.

11. Matsuo, Y. (1984) : Futher studies of the use of cyclohexiмide for cultivating *Mycobacterium leprae-murium* in cell culture. *Microbiol. Immunol.* 28 : 1093–1098.

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